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Coproantibodies to Milk: Identification by Radioimmunoelectrophoresis[1]

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Precipitating antibodies against cow's milk, as well as high titers of hemagglutinins, have been found in the feces of subjects suffering from milk sensitivity or celiac disease (1, 2). Low titers of hemagglutinating, but not precipitating, coproantibodies were found in the majority of normal infants or patients with other diseases (2).

There is some direct and indirect evidence that coproantibodies to viruses consist mainly of immunoglobulin A (3–5). The data of Katz *et al.* (1, 6) suggest that this is also the case with coproantibodies to soluble proteins. The use of the radioimmunoelectrophoresis (RIEP) technique proved in the present study to be useful for direct identification of the immunoglobulin types which make up the coproantibodies to milk.

The sera and fecal supernatants examined in the present study were obtained from infants (aged 3 to 12 months) with gastrointestinal milk sensitivity. These infants suffered from diarrhea and vomiting that ceased after elimination of cow's milk from the diet and the diagnosis was confirmed by three challenges with milk. These cases have been described elsewhere (2, 7). Fecal supernatants were prepared by centrifugation of the fecal samples at 12,000 × G for 30 min in the cold. The sera and fecal supernatants were tested by the RIEP technique as described in detail by Yagi *et al.* (8). Goat antiserum against human globulins prepared by us or monospecific antisera against individual immunoglobulins (Hyland) were used for development of precipitation lines and were followed by radioiodinated (^{125}I)-milk from which fat and casein were removed (7). The autographs were developed after exposure for 14 days.

The immunoelectrophoresis (IEP) and the corresponding RIEP reactions of sera and fecal supernatants from three milk-sensitive cases are shown in Figure 1. The fecal supernatants contained relatively more IgA but less IgG than did the corresponding sera. IgM was also detected in the fecal supernatant of case 2. Specific antibody activity, demonstrated by the RIEP test, was shown in the fecal samples to reside mainly in the IgA fraction, with smaller amounts of antibody of the IgG type. In the sera, however, intense milk binding was found in the IgG zone with only small amounts of antibodies of the IgA type in cases 1 and 2. The fecal supernatant of case 2 when tested against monospecific goat antihuman IgM antiserum yielded a more intense precipitation line in the IEP test, and moderate antigen binding was noted on the autoradiograph (Fig. 2). Other supernatants gave negative results with anti-IgM.

Fecal supernatants from infants with other diseases, as well as from normal controls, showed very little or no antibody against milk by the RIEP test (2). In all positive cases the antigen-binding activity resided in the IgA precipitation line. Serum antibodies to milk, however, in these subjects also were mainly of the IgG type.

Sera and fecal supernatants were tested by RIEP also with purified bovine serum albumin and bovine γ globulin. Both antigens gave positive RIEP reactions, which correlated well with the reactions obtained by whole milk.

The intensity of antibody activity in serum and fecal supernatants is not parallel in most cases. This is shown in Figure 1, in which case 1 has intense antibody activity in the serum with little activity in the feces, while the opposite situation exists in case 3.

The data lend further support to the assumption that the antibody systems of the serum and feces are independent. The two systems were shown to differ in both their intensity and immunoglobulin composition. Furthermore, se-

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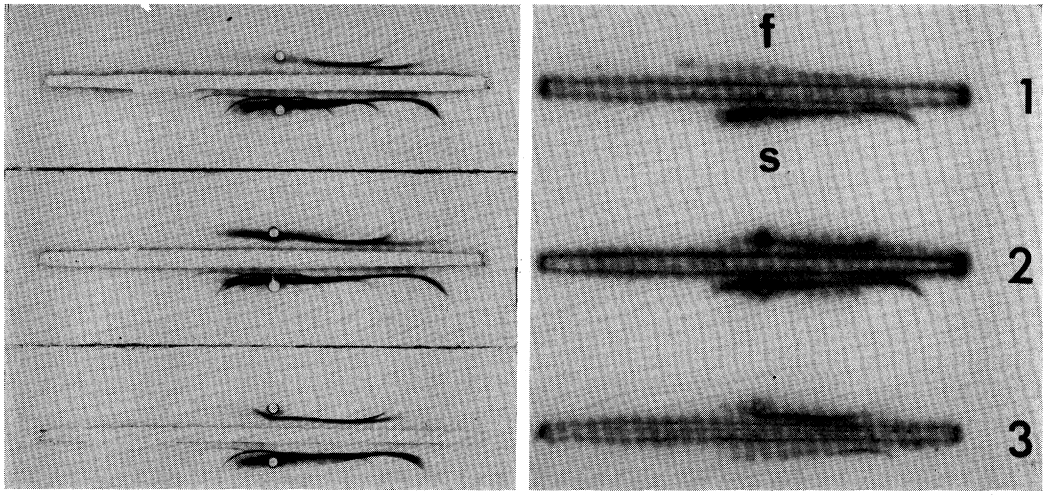

Figure 1. Immunoelectrophoresis (IEP, *left*) and the corresponding autoradiographs (RIEP, *right*) of fecal supernatants (*f*) and sera (*s*) from three infants (*1*, *2*, *3*) with milk sensitivity. Goat anti-human globulin (33% saturated ammonium sulfate precipitate) was put into the troughs and was followed by ^{125}I-whole cow's milk.

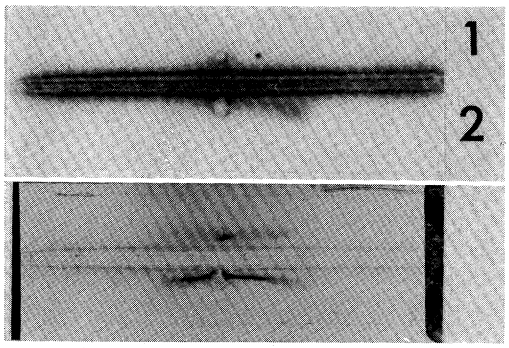

Figure 2. RIEP (*top*) and IEP (*bottom*) reactions of fecal supernatants from cases *1* and *2*, against goat anti-human IgM and ^{125}I-whole cow's milk.

cretory IgA, which makes up most of the fecal antibody, is known to be produced locally and to contain an extra antigenic piece (reviewed in reference 9), and indeed, the serum and fecal IgA differed in their IEP migration patterns (Fig. 1). Fecal IgG and IgM, however, may originate also in the serum, especially in cases with gastrointestinal sensitivity, in which the intestinal wall is damaged and exudation of serum proteins takes place (10).

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